unliganded gating, and the mutation αW149F reduces the ACh affinity of **C** only by 13-fold, but of **O** by 190-fold. Rate-equilibrium free energy relationships for different regions of the protein show similar slopes (Φ-values) for un- vs. diliganded gating. The mechanisms of the gating conformational change and of desensitization are similar with and without ligands at the transmitter binding sites.

864-Pos Board B743

Detection and Trapping of Elusive Priming Intermediates Towards Open Nicotinic Receptor Channel

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Acetylcholine receptors (AChRs) mediate rapid synaptic transmission by transducing a chemical signal into an electrical impulse. Transduction comprises binding of agonist followed by opening of the AChR ion channel, and in the classical view both processes depend on the agonist. However previous studies suggest the ultimate channel opening step is agonist-independent[1,2], and is preceded by a priming step facilitated by the agonist[3]. Here, by studying mutant AChRs, we detect two such priming steps; the first generates a closed state that elicits brief openings, and the second generates a closed state that elicits long-lived openings. Long-lived openings and the associated priming step are detected in the absence of agonist and in its presence, and show identical kinetics under each condition. By covalently locking the agonist binding sites in the bound conformation, we show that each site initiates a priming step. Thus a change in binding site conformation primes the AChR for channel opening in a process that determines the maximum response to agonist and functional consequences of disease-causing mutations.

865-Pos Board B744

Single Channel Current Through Nicotinic Receptor Produced By Closure Of The Binding Site C-loop

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We investigated the initial coupling of agonist binding to channel gating of the nicotinic acetylcholine receptor (nAChR) using Targeted Molecular Dynamics (TMD) simulation. Following TMD to accelerate closure of the C-loops at the agonist binding sites, the region of the pore that passes through the cell membrane expands. To determine whether the structural changes in the pore result in ion conduction, we used a coarse-grained ion conduction simulator, called Biology Boltzmann Transportor Monte Carlo (BioMOCA) simulation, and applied it to two structural frames taken from before and after the TMD simulation. The structural model of the pre-TMD simulation represents the channel in the proposed ''resting'' state, whereas the model of the post-TMD simulation represents the proposed "active" state. Under external voltage biases, the channel in the ''active'' state was permeable to cations. Our simulated ion conductance approaches that obtained experimentally and recapitulates several known functional properties of the nAChR. Thus, closure of the C-loop triggers a structural change in the channel pore that is sufficient to account for the open channel current. This approach of applying BioMOCA in computational studies of ion channels can be used to uncover the binding to gating transduction mechanism and the structural bases for ion selection and translocation.

866-Pos Board B745

Electrical Fingerprinting Reveals Agonist Binding Sites Required for Activation of Homo-pentameric Cys-loop Receptors

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Ancestral neurotransmitter Cys-loop receptors were homo-pentamers harboring five identical agonist binding sites but most present day receptors are hetero-pentamers with only two binding sites. To understand why Cys-loop receptors evolved to utilize fewer than five binding sites, we disabled different numbers of sites and developed a method to monitor lifetimes of individual active receptors and the corresponding number of functional binding sites. We find that maximal open-channel lifetime is achieved when the neurotransmitter occupies three non-consecutive binding sites. Occupancy of one site allows receptor activation, although the open state is unstable; occupancy of two non-consecutive sites produces a much longer-lived open state appropriate for efficient activation. However, occupancy of a third site further increases channel lifetime, thus providing optimal stabilization of the active state. Maximal activation of homomeric receptors by agonist occupancy of less than the five potential sites enhances the rate of channel opening and increases agonist sensitivity.
The results reveal that allosteric requirements dictated the number and location of the agonist binding sites, and provide an indispensable framework for further progress in drug design.

867-Pos Board B746

Photoaffinity Labeling the Agonist Binding Sites of nAChRs with [^{3}H]Epibatidine

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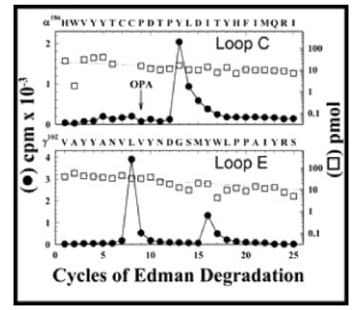
Neuronal nAChR subtype-selective agonists have potential therapeutic uses in many neurological disorders. Determination of structural aspects unique to individual nAChR agonist binding sites (ABS) is important to the development of nAChR subtype-selective agonists/ligands. To this end, we photolabeled *Torpedo*, α4β2 and α4β4 nAChRs with [^{3}H]Epibatidine. [^{3}H]Epibatidine binds to α4β2 and α4β4 nAChRs with high affinity (10-200 pM) and binds with similar affinity at the α1-γ and α1-δ ABS of the *Torpedo* nAChR (~11 nM). At the subunit level, [^{3}H]Epibatidine photoincorporated into the principal component of the ABS (α1 and α4 subunits) and the complementary component of the ABS in γ and β4 subunits but not in the δ or β2 subunits. Since little is known about the photochemistry of [^{3}H]Epibatidine and the stability of UV-induced [^{3}H]Epibatidine-amino acid adducts under Edman degradation conditions, we first established the merit of [^{3}H]Epibatidine as a photoaffinity probe by determining sites of [^{3}H]Epibatidine labeling in the *Torpedo* nAChR. The principal sites of labeling were αTyr^{198} within Loop C and γLeu^{109} and γTyr^{117} within Loop E of ABS (see figure). Studies are currently underway to identify the sites of labeling within the α4 and β4 subunits.



868-Pos Board B747

Hyperfine Splitting Trends in the EPR Spectra of M2δ in Aligned Phospholipid Bilayers

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In Nuclear Magnetic Resonance (NMR) spectroscopic techniques, polarization index slant angle (PISA) wheels, dipolar waves. and residual dipolar coupling waves, demonstrate the relation between the secondary periodic structure of α helices and their magnetic properties. Based on very many of the same principles as NMR, it is anticipated that similar trends will be evident in the information obtained from Electron Paramagnetic Resonance (EPR) studies of spin-labeled α-helical membrane proteins incorporated into aligned lipid bilayers. Towards this end, we have proposed that a rigid spin- labeled transmembrane α-helix exhibits a sinusoidal periodicity in the EPR specific hyperfine splitting values obtained for consecutively labeled residues of the peptide. We have shown that this can be mathematically related to the helical tilt angle at which it is oriented within the membrane and the corresponding static magnetic field. This phenomenon is demonstrated using the M2δ pore lining peptide of the nicotinic acetylcholine (AChR) receptor. Also, the effect of environmental conditions such as motional averaging caused by rotation of the M2δ helix within the membrane. These experimental results are evidence of how a theoretical model can be used to determine the helical tilt angle of M2δ, and by extrapolation, the helical tilt angle of any other membrane protein - verifying a relatively simple, but powerful method of extracting crucial topological information from minimal experimental EPR data.

869-Pos Board B748

Examining the Structure of the Neuronal a4b2 nAChR Transmembrane Domain by Photoaffinity Labeling

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The ability to purify neuronal nAChRs in large quantities allows the use of photoaffinity labeling to study their structure. To characterize the structure of the transmembrane domain of the α4β2 nAChR, we used [^{3}H]chlorpromazine, which has been used to identify amino acids in the *Torpedo* nAChR ion channel, and [^{3}H]TDBzl-etomidate, which acts as a *Torpedo* nAChR positive allosteric modulator by binding at a novel site within the transmembrane domain at the interface between the γ and α subunits. In the presence of agonist, [^{3}H]chlorpromazine and [^{3}H]TDBzl-etomidate incorporated into α4 and β2 subunits